

Review Article

Relating SMCHD1 structure to its function in epigenetic silencing

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The structural maintenance of chromosomes hinge domain containing protein 1 (SMCHD1) is a large multidomain protein involved in epigenetic gene silencing. Variations in the *SMCHD1* gene are associated with two debilitating human disorders, facioscapulo-humeral muscular dystrophy (FSHD) and Bosma arhinia microphthalmia syndrome (BAMS). Failure of SMCHD1 to silence the *D4Z4* macro-repeat array causes FSHD, yet the consequences on gene silencing of SMCHD1 variations associated with BAMS are currently unknown. Despite the interest due to these roles, our understanding of the SMCHD1 protein is in its infancy. Most knowledge of SMCHD1 function is based on its similarity to the structural maintenance of chromosomes (SMC) proteins, such as cohesin and condensin. SMC proteins and SMCHD1 share similar domain organisation and affect chromatin conformation. However, there are important differences between the domain architectures of SMC proteins and SMCHD1, which distinguish SMCHD1 as a non-canonical member of the family. In the last year, the crystal structures of the two key domains crucial to SMCHD1 function, the ATPase and hinge domains, have emerged. These structures reveal new insights into how SMCHD1 may bind and regulate chromatin structure, and address how amino acid variations in SMCHD1 may contribute to BAMS and FSHD. Here, we contrast SMCHD1 with canonical SMC proteins, and relate the ATPase and hinge domain structures to their roles in SMCHD1-mediated epigenetic silencing and disease.

Introduction

The structural maintenance of chromosomes hinge domain containing protein 1 (SMCHD1) is an epigenetic regulator that controls gene expression at selective sites across the genome [1]. While its initial discovery revealed that *Smchd1*¹ is critical in the process of X-chromosome inactivation and thereby essential in female embryo viability [2], numerous studies have now recognised its role in regulating the expression of various autosomal gene clusters such as *Pcdh* and *HoxB*, in addition to monoallelically expressed targets such as selected genes within the *Snrpn* cluster [3–7]. The exact underlying mechanism remains unknown, but experimental evidence suggests that *Smchd1* is involved in the maintenance of long range chromatin looping such that it limits promoter–enhancer interactions and therefore creates a transcriptionally repressive environment [4,8,9].

Importantly, heterozygous *SMCHD1* variants are associated with autosomal dominant facioscapulo-humeral muscular dystrophy (FSHD) and the rare craniofacial disorder Bosma arhinia microphthalmia syndrome (BAMS) (Table 1) [10–12]. However, the mechanisms by which pathogenic *SMCHD1* variants lead to different clinical disorders are not fully understood. The reported pathogenic variants associated with the two conditions do not typically overlap and the resulting phenotypic outcomes are

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¹Lower case for *Smchd1* indicates mouse isoform in place of upper case (SMCHD1) for the human isoform.

Table 1. SMCHD1 single nucleotide polymorphisms (SNPs) resulting in missense mutations described in patients with facioscapulohumeral muscular dystrophy type 2 (FSHD2) and Bosma arhinia microphthalmia syndrome (BAMS) Part 1 of 2

Mutation	SMCHD1 domain	Associated disease	Pubmed ID
Arg34Pro	UBL	FSHD2	31243061
Asn104Ser	UBL	FSHD2	31243061
Leu107Pro	UBL-ATPase linker	FSHD2, BAMS	31243061, 29980640, 28067909
Ala110Thr	UBL-ATPase linker	FSHD2	31243061, 25370034
Met129Arg	ATPase	BAMS	31243061
Met129Lys	ATPase	BAMS	31243061, 28067909
Ala134Ser	ATPase	BAMS	31243061, 28067911
Ser135Asn	ATPase	BAMS	31243061, 28067909, 28067911
Ser135Cys	ATPase	BAMS	31243061, 28067909, 28067911, 30698748
Ser135Ile	ATPase	BAMS	31243061, 28067909, 28067911
Glu136Asp	ATPase	BAMS	31243061, 28067909
Glu136Gly	ATPase	BAMS	31243061, 28067911, 30698748
Gly137Glu	ATPase	FSHD2, BAMS	31243061, 28067909, 25256356
Asn139His	ATPase	BAMS	31243061, 28067909
Leu141Phe	ATPase	BAMS	31243061, 28067909, 28067911
Asp150His	ATPase	FSHD2	31243061
Phe171Val	ATPase	BAMS	31243061, 28067909
Gly188Arg	ATPase	FSHD2	31243061
Met189Val	ATPase	FSHD2	31243061
Gln193Pro	ATPase	FSHD2	30698748
Leu194Phe	ATPase	FSHD2	31243061, 25256356
Lys204Glu	ATPase	FSHD2	31243061, 29980640
Ala242Gly	ATPase	BAMS	31243061, 28067909
Ala242Thr	ATPase	FSHD2	31243061, 29980640
His263Asp	ATPase	FSHD2	31243061, 25256356
Glu264Lys	ATPase	FSHD2	31243061
Tyr283Cys	ATPase	FSHD2	31243061, 27061275
Trp324Ser	ATPase	BAMS	31243061, 28067911
Arg344Gln	ATPase	FSHD2	31243061, 29980640
Gln345Arg	ATPase	BAMS	31243061, 28067909
His348Arg	ATPase	BAMS	31243061, 28067909, 28067911
Tyr353Cys	ATPase	FSHD2	31243061, 23143600
Gln400Leu	Transducer	BAMS	31243061, 28067909
Asp420Val	Transducer	BAMS	31243061, 28067909, 28067911, 30698748
Gly425Arg	Transducer	FSHD2	31243061, 25256356
Arg428Cys	Transducer	FSHD2	31243061
Glu473Gln	Transducer	BAMS	31243061, 28067909
Gly478Glu	Transducer	FSHD2	31243061, 25370034
Arg479Pro	Transducer	FSHD2	31243061, 23143600
Arg479Leu	Transducer	FSHD2	31243061, 28744936
Arg479Gln	Transducer	FSHD2	31243061

Continued

Table 1. SMCHD1 single nucleotide polymorphisms (SNPs) resulting in missense mutations described in patients with facioscapulohumeral muscular dystrophy type 2 (FSHD2) and Bosma arhinia microphthalmia syndrome (BAMS) Part 2 of 2

Mutation	SMCHD1 domain	Associated disease	Pubmed ID
Cys492Arg	Transducer	FSHD2	31243061, 23143600
Lys518Glu	Transducer	BAMS	31243061, 28067911
Phe519Ser	Transducer	FSHD2	31243061, 29980640
Thr523Lys	Transducer	BAMS	31243061, 28067909
Asn524Ser	Transducer	BAMS	31243061, 28067909
Thr527Met	Transducer	FSHD2	31243061, 24075187
Gln551Arg	Transducer	FSHD2	31243061
Arg552Gln	Transducer	BAMS	31243061, 28067909, 28067911
Trp596Gly	linker (strand prediction)	FSHD2	31243061
Val615Asp	linker (strand prediction)	FSHD2	31243061, 25370034
Pro622Leu	linker (helical prediction)	FSHD2	31243061
Val641Leu	linker (strand prediction)	FSHD2	31243061
Pro690Ser	linker (loop prediction)	FSHD2	31243061, 23143600
Leu748Pro	linker (strand prediction)	FSHD2	31243061, 25256356
Tyr774Cys	linker (loop prediction)	FSHD2	31243061
Asp849Asn	linker (strand prediction)	FSHD2	31243061, 23143600
Leu923Pro	linker (strand prediction)	FSHD2	31243061
Leu978His	linker (loop prediction)	FSHD2	31243061
Tyr981Asp	linker (strand prediction)	FSHD2	31243061, 27153398
Gly1063Arg	linker (disorder prediction)	FSHD2	31243061
Leu1108Pro	linker (disorder prediction)	FSHD2	31243061
Val1114Ile	linker (disorder prediction)	FSHD2	31243061
Val1271Leu	linker (strand prediction)	FSHD2	31243061
Ile1300Lys	linker (disorder prediction)	FSHD2	31243061
Gln1463Pro	linker (strand prediction)	FSHD2	31243061, 25370034
Met1468Ile	linker (strand prediction)	FSHD2	31243061, 25256356
Pro1485Leu	linker (disorder prediction)	FSHD2	31243061, 25370034
Phe1554Ser	linker (helical prediction)	FSHD2	31243061, 23143600
Asp1750Gly	SMC hinge	FSHD2	31243061
Asp1750Val	SMC hinge	FSHD2	31243061
Tyr1846Cys	SMC hinge	FSHD2	31243061
Arg1866Gly	SMC hinge	FSHD2	31243061, 27153398
Arg1866Gln	SMC hinge	FSHD2	31243061

Mutations are listed according to sequence and the associated SMCHD1 domain indicated. For the linker region there is no available structure and secondary structure prediction from the Phyre2 server is indicated [63]. Pubmed IDs for papers that describe the SNPs are indicated [10–12,48,62,64–69].

entirely distinctive, suggesting different molecular mechanisms are at play. SMCHD1 is a very large and flexible dimer of ~250 kDa protomers, which presents a major challenge in understanding SMCHD1's mechanism of action via structural approaches. Only one study to date has revealed low-resolution images of full-length Smchd1 via negative stain electron microscopy [13]. In the last year, X-ray crystallography has provided the first high-resolution structures of its two known functional regions: the N-terminal ATPase and C-terminal hinge domains [14,15].

SMCHD1 is a non-canonical SMC protein

The structural maintenance of chromosomes (SMC) family of proteins are key organisers of chromatin architecture in all living organisms [16]. SMCHD1 is considered a non-canonical member of the SMC family owing to differences in its type of ATPase domain and in its overall linear domain architecture [14,17–21] (Figure 1A,B). SMC protein complexes function via highly conserved mechanisms owing to their essential roles, such as mediating chromosome conformation throughout the cell cycle. In mammals, each of the various functional complexes comprise of a heterodimeric pair of SMC subunits, such as SMC1 and SMC3, or SMC2 and SMC4, which are the core components of cohesin and condensin, respectively [17–19,21]. It should be noted that whilst prokaryotes have homodimeric SMC proteins, in this review we focus on mammalian proteins as there are no prokaryotic equivalents of SMCHD1. Each SMC monomer has three regions: the ABC-type ATPase domain that is split between the N- and C- termini, the DNA-interacting SMC hinge domain and an extended coiled-coil linker region that bridges the two domains (Figure 1B) [21–23]. The canonical SMC primary sequence starts with the Walker A motif from the ABC-type ATPase, followed by a N-terminal α -helix that links to the SMC hinge domain. At the C-terminus of the SMC hinge there is another α -helix, which forms an antiparallel coiled-coil with the N-terminal α -helix. This coiled-coil folds the protein back on itself allowing the C-terminal Walker B motif to dimerise with the N-terminal Walker A motif to form a functional ABC-type ATPase domain.

SMCHD1 also has a SMC hinge domain, however, in contrast with canonical SMC proteins, it is located at the protein's C-terminus (Figure 1A) [1,2,24]. A characteristic of canonical SMC hinge domains is the GX_6GX_3GG motif that lies at the hinge domain dimer interface. In SMCHD1, this sequence is one amino acid shorter, GX_6GX_2GG , which leads to altered structure in this region [15]. Furthermore, the SMCHD1 ATPase has a GHKL-type architecture and is located uniquely at the N-terminus instead of an ABC-type ATPase split between the N- and C- termini (Figure 1A,B) [13,14,20]. Between the ATPase and SMC hinge there is a long linker region spanning approximately 1200 amino acids. In canonical SMC proteins, this region is predominantly α -helical, but for SMCHD1 it is predicted to consist of β -strands with only the last 200 amino acids, proximal to the SMC hinge, predicted to be α -helical (Figure 1A,B). Finally, the C-terminal segment in SMCHD1 is predicted to contain a relatively short α -helix spanning approximately 100 amino acids, compared with approximately 300 amino acids for the canonical SMC proteins [25,26]. In mammals, the canonical SMC proteins heterodimerise through interactions primarily at the hinge domain interface with some interactions at the ATPase region [17–19,21]. In contrast, SMCHD1 forms a homodimer at the C-terminus through its SMC hinge domain (Figure 1A) [24]. SMCHD1 can also homodimerise through its N-terminal ATPase region (Figures 1A and 2A). This dimerisation occurs via exchange of a ubiquitin-like (UBL) domain from one protomer to the other; this UBL domain is not present in the canonical SMC proteins (Figure 1A and 2A) [14]. In addition, evidence from small-angle X-ray scattering (SAXS) and electron microscopy indicates that SMCHD1 α -helices form N- to N- and C- to C- coiled-coil pairings [13,24], providing an additional region for SMCHD1 homodimerisation.

The SMC heterodimers form a closed ring structure that is proposed to topologically entrap or encircle DNA [27,28] (Figure 1C,D). Despite being well-studied, their exact molecular mechanism is not established. The 'loop extrusion' model is the most widely accepted mechanism that describes the functions of cohesin and condensin. This model proposes they push chromatin through their central coiled-coil ring structure to form chromatin loops in an ATP-dependent manner [19,29,30]. This energy-dependent process drives loop elongation, where one or both DNA interaction sites translocate away from each other, leading to the formation of chromatin loops (Figure 1C,D). The association of cohesin with chromatin is additionally dependent on the transcription factor CCCTC-binding factor (CTCF), which acts as a barrier to the loop extrusion process by stabilising cohesin at CTCF-binding sites and creating the base of the established DNA loop [31,32]. This mechanism facilitates promoter–enhancer interactions between distal regions of the genome to help establish functional domains that are required for transcriptional regulation. The loop extrusion model has recently been demonstrated *in vitro* for both yeast condensin [33,34] and human cohesin [35,36], where the process was visualised via single-molecule live imaging techniques using surface-tethered DNA. While yeast condensin was observed to undergo the predicted topological entrapment of DNA where both strands are embraced by its ring-like structure, human cohesin appeared to instead interact with DNA pseudo-topologically or non-topologically; therefore, embracing either one strand of DNA only or not encircling DNA entirely [35,36]. Precisely how SMC

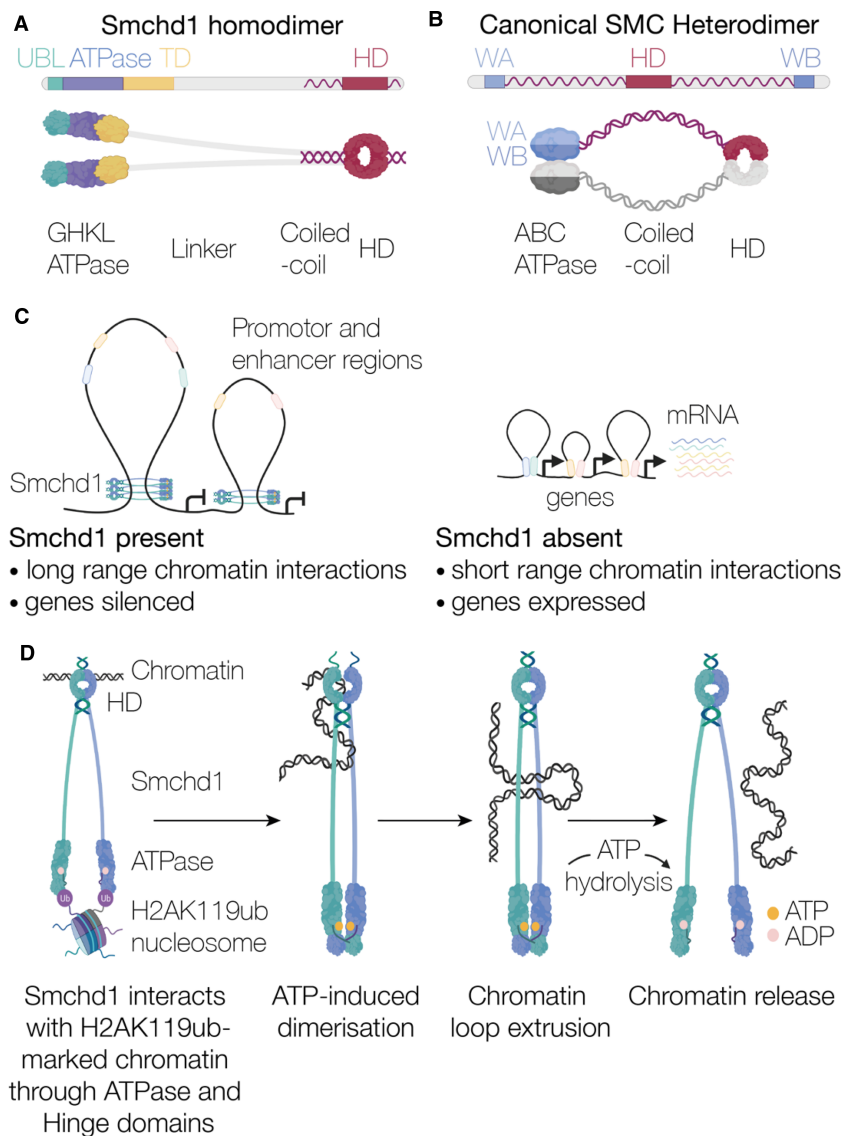


Figure 1. Overall structure and function of Smchd1.

Schematic comparing the similarities and differences between (A) Smchd1 and (B) a canonical SMC protein. (C) Smchd1 causes an increase in long range chromatin interactions silencing gene expression. (D) Hypothetical model of Smchd1 engaging with chromatin to facilitate long range DNA interactions controlled by ATP hydrolysis.

complexes use their ATPase activity to facilitate chromosome structure rearrangements remains uncertain. However, common to all proposed mechanisms, SMC proteins use ATP hydrolysis to change conformation and entrap DNA, and this ultimately drives dynamic loop formation [16,33,37,38].

There are clear similarities between SMCHD1 and canonical SMC proteins. However, differences in gene architecture and domain organisation between SMCHD1 and canonical SMC proteins suggest that functional differences may also be present. Experimental evidence suggests that SMCHD1 retains the ability to alter chromatin structure as part of its mechanism of action, similar to canonical SMC proteins [4,8,9]. But, while canonical SMC proteins are known to assemble into functional protein complexes by forming heterodimers, SMCHD1 is only known to form homodimers. This feature along with the presence of a GHKL-type ATPase domain in SMCHD1 as opposed to ABC-type ATPases (Figure 1A,B) may indicate that it is more similar to the MORC family of proteins, which are also part of the GHKL superfamily [39]. The MORC family use ATP-binding to control dimerisation events and entrap DNA [40], forming chromatin loops by compaction, as opposed to the active loop extrusion proposed for canonical SMC proteins [33,35,36].

ATPase domain

SMCHD1 has a GHKL-type ATPase domain, a functionally diverse protein superfamily that is named for the archetypal members, Gyrase B, Hsp90 histidine kinase and MutL [20,41]. GHKL ATPases typically consist of a Bergerat fold which describes an α/β sandwich that consists of a four-stranded β -sheet and three α -helices, in addition to a unique feature — a long flexible loop known as the ATP-lid. This loop is highly variable across members of the GHKL superfamily. Different sequences and conformations among the ATP-lid distinguish different GHKL proteins, yet they all hold a conserved role in ATP-binding which suggests their functional importance. The recently solved crystal structure of a SMCHD1 ATPase construct (residues 25–580) revealed the Bergerat fold as the catalytic domain (residues 110–395) (Figure 2A) [14]. Pedersen *et al.* also identified a novel UBL domain at the N-terminus (residues 25–110) that undergoes a domain-swapping event between two SMCHD1 monomers via an N-terminal β -strand (residues 110–120) (Figure 2A). This is the first study to describe the dimerisation of SMCHD1's ATPase domain, which is not an unexpected finding considering many GHKL ATPases sustain the ability to homodimerise [41]. Additionally, the obligate homodimerisation of SMCHD1's hinge domain reflects potential contacts at the opposing end of the protein to where the N-terminal ATPase domain resides. This study revealed that SMCHD1 dimerisation requires not only the UBL domain, but also the presence of ATP, in addition to the transducer domain that abuts the C-terminus of the ATPase domain (Figure 2A). Surprisingly, this structure did not reveal any interface contacts between SMCHD1 monomers at the transducer domain; dimerisation appears to instead create a cavity between the transducer domains of two monomers. An explanation for the inability of SMCHD1 to dimerise upon absence of the transducer domains was not postulated. The distal C-terminal region commonly serves as a site of homodimerisation across members of the GHKL family [42–44]. It would, therefore, be interesting to further investigate whether extending the SMCHD1 ATPase construct at the C-terminus may reveal contacts between the transducer domains.

Despite providing valuable insights into the molecular structure of SMCHD1, it is important to note this structure used a catalytically inactive point mutant of the ATPase domain [3]. The E147A mutation introduced was previously shown to completely abolish the ATPase activity of Smchd1 [20], as the glutamic acid is a conserved residue across members of the GHKL superfamily that is indispensable for the ATP hydrolysis step. Because this crystal structure was solved in the presence of ATP, it is presumed the E147A mutant retains the

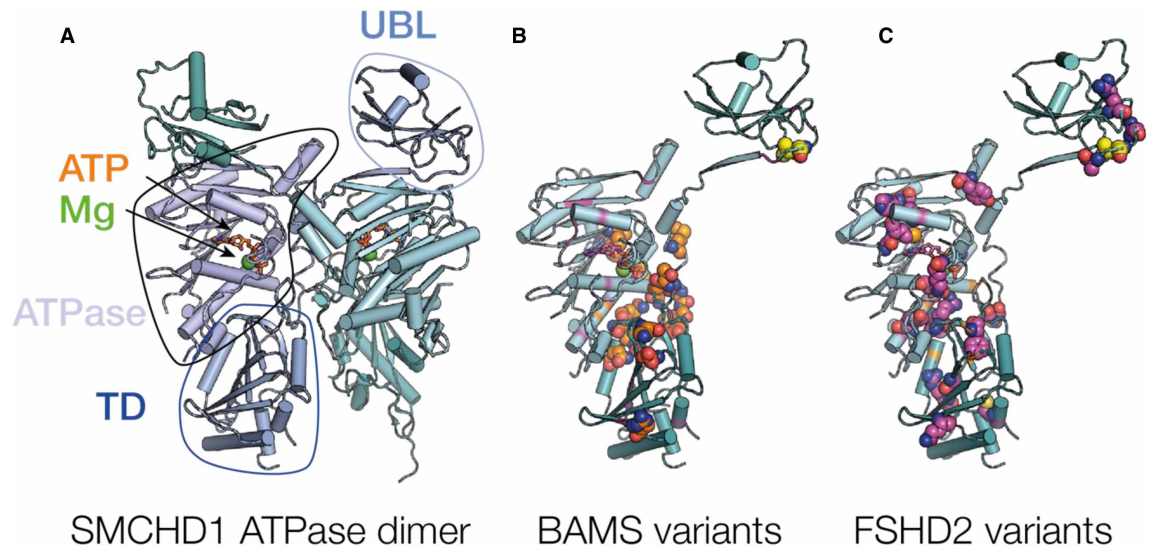


Figure 2. The SMCHD1 ATPase domain.

(A) Representation of the SMCHD1 GHKL ATPase region (PDB ID 6MW7) [14]. The image shows strand-swapped ubiquitin-like (UBL) domain (circled) bound to the GHKL ATPase from the opposing monomer. The ATPase, ATP and magnesium (Mg) binding sites, and transducer domains (TD) from the same protomer are also circled and labelled. (B,C) The locations of missense variants within the ATPase region that are associated with (B) BAMS (orange) and (C) FSHD2 (magenta) displayed on one monomer from the dimer. Missense variations associated with both diseases are shown in yellow.

ability to bind, but not hydrolyse, ATP. Interestingly, dimerisation triggered by ATP-binding is a common feature among GHKL ATPases [40,45–47]. This is closely followed by the closing of the ATP-lid over the active site to allow ATP hydrolysis and subsequent dissociation of the dimer. If the E147A mutant is trapped in the ATP-bound state, this phenomenon likely justifies a preferential dimerisation over wild-type SMCHD1. This idea is further supported by native PAGE analyses where a proportion of the E147A variant of SMCHD1 migrates as a higher molecular mass species under native conditions, which was interpreted as a dimer. Surprisingly, the wild-type or any other SMCHD1 variant tested remained largely monomeric. However, these results contrast chemical cross-linking experiments performed for the corresponding SMCHD1 variants, which suggest that all except FSHD2-related mutants exhibit some capacity to dimerise [14]. It, therefore, remains of outstanding interest whether the wild-type counterpart adopts a similar dimeric conformation to the E147A variant, prompting more in-depth biophysical experiments to establish dimerisation parameters for both wild-type and variant forms of SMCHD1.

All *SMCHD1* variations found in BAMS patients that have been identified to date map to the N-terminal region, in addition to numerous FSHD2-associated variations in the same region, highlighting the overall importance of the N-terminal region in SMCHD1's function (Figure 2B,C and Table 1). One of the most intriguing aspects of unveiling an atomic structure of SMCHD1's ATPase domain was the ability to spatially map pathogenic variants to help provide a better understanding in how these may alter SMCHD1's function as an epigenetic regulator. Largely, it seems disease variants do not cluster in specific regions of the ATPase domain, although there seems to be a hotspot for BAMS-associated mutations located in a loop region of SMCHD1 that is situated at the dimer interface, suggesting that BAMS-associated variants possibly alter SMCHD1 dimerisation (Figure 2B,C). Prior to the release of the first crystal structure of SMCHD1's ATPase domain, a separate study mapped the location of FSHD2 and BAMS variants in SMCHD1 based on the crystal structure of the GHKL ATPase TRAP1 [48]. Their findings suggested that FSHD2 variants are almost exclusively located around the ATP-binding site, whereas the majority of BAMS variants localise to a loop region within the dimer interface, consistent with the published structure of SMCHD1. Pedersen *et al.* [14] briefly explored the dimerisation properties of SMCHD1 variants via native PAGE and cross-linking experiments, and concluded that dimerisation was preserved in BAMS-associated mutants but greatly reduced in FSHD2-associated mutants. This raises the possibility that *SMCHD1* variations in FSHD2 patients may impact ATP-binding and dimerisation in the N-terminal region, which would be expected to diminish SMCHD1 function. Conversely, it remains to be explored whether variations identified in BAMS patients might influence other functions of SMCHD1, such as chromatin interactions or recruitment of potential protein interactors. While ATPase domain catalytic activity appears important for normal SMCHD1 function, it is compromised in some FSHD2 patients [49]. Additional FSHD2-associated variations have been identified throughout the *SMCHD1* gene and are not limited to the ATPase region. Each of these variations prevents SMCHD1 silencing the *D4Z4* macro-repeat array in FSHD2 patients. Therefore, defective ATPase dimerisation is one explanation for SMCHD1's loss of function in FSHD2, but there are likely multiple contributing factors that require examination in the context of the full-length protein.

Hinge domain

The hinge domain of SMCHD1 is located at the C-terminus of the protein. The hinge domain forms the principal interface for the assembly of SMCHD1 dimers and can interact with chromatin. We recently solved the structure of the mouse *Smchd1* hinge domain, providing the first example of a homodimeric mammalian SMC hinge domain (Figure 3A) [15]. The *Smchd1* hinge domain homodimer comprised two protomers related by rotational symmetry, with each monomer containing two pseudo-symmetrical subdomains bridged by an inter-subdomain linker region (Figure 3A). This structure shared most of the secondary structure features present in the canonical SMC hinge domains, however, there were distinct differences. The closest published structural homologue was from the extremophile bacteria *Thermotoga maritima* (Figure 3B,C) and the closest mammalian structure was from human SMC4, varying by a root mean squared deviation of 3.4 and 3.8 Å, respectively [28,50]. This large deviation in architecture is due to a change in the angle between the two subdomains. Hinge domains have two dimerisation interfaces between subdomain I and subdomain II of opposing protomers. The dimerisation interface in *Smchd1* contains unique hydrogen bonding, hydrophobic and salt bridge interactions that facilitate its homodimerisation and preclude its heterodimerisation with the other mammalian SMC proteins (Figure 3D). An interesting feature of SMC hinge domains is an extended β -sheet that bridges across the dimerisation interface, typically comprising three strands from subdomain I on one protomer and

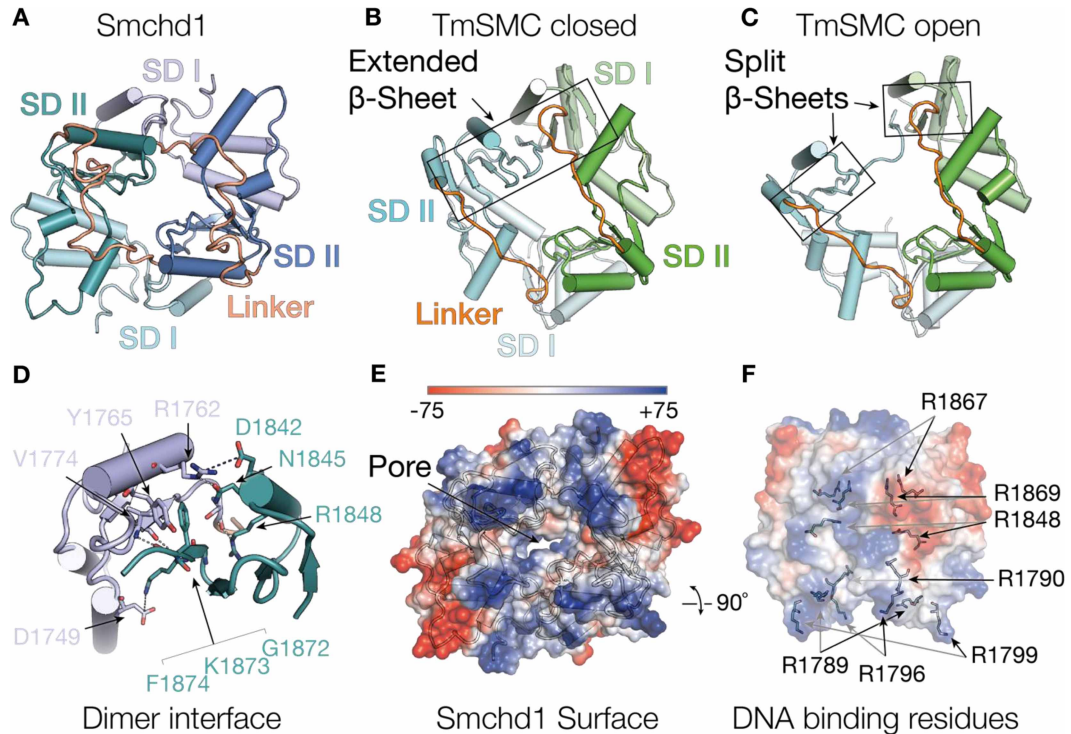


Figure 3. The Smchd1 Hinge domain.

(A) Structure of the mouse Smchd1 hinge domain showing cartoon representation of the two protomers (green and blue) in the homodimer indicating subdomain I (SD I, pale colours) and subdomain II (SD II, dark colours) and subdomain linker region (orange). (B,C) Structures of the *Thermotoga maritima* SMC hinge domain showing both protomers (cyan and green) in the dimers, subdomain I (SD I, pale colours) and subdomain II (SD II, dark colours) and subdomain linker region (orange) displayed. (B) shows a closed hinge domain conformation with an extended β -sheet and (C) open conformation with a split β -sheet at one dimerisation interface. Structures are displayed in the same orientation as Smchd1, aligning on subdomain II of the blue and green protomers from smchd1 and *T. maritima* SMC hinge domains, respectively. (D) Cartoon representation of the Smchd1 HD dimerisation interface between SD I (pale) of one protomer to SDII from the opposing protomer (colouring as in panel (A)). Key residues involved in the interaction are indicated in stick representation with hydrogen bonding and electrostatic interactions indicated with dashed lines. (E) Surface electrostatic representation of the Smchd1 HD indicating the surface exposed positive region in the interdomain linker region. (F) Surface representation of the Smchd1 HD showing positively charged arginine residues involved in binding DNA. These include surface exposed residues from the interdomain linker region (R1789, R1796, R1799) and residues buried in the dimer pore (R1790, R1848, R1869), and R1867, which is substituted to glycine in an FSHD patient kindred. Black and grey arrows indicate residues from opposing protomers in the Smchd1 dimer.

five strands from subdomain II on the opposing protomer (Figure 3A–C) [28,51–54]. Published structures of the canonical SMC hinge domains adopt either open or closed conformations, (Figure 3A–C). In a closed conformation both dimerisation interfaces are intact showing two extended β -sheets (Figure 3B) [28,50]. In an open conformation, one dimerisation interface is lost resulting in one of the extended β -sheets splitting in half to form two discrete β -sheets (Figure 3C) [28,53,54]. This splitting of the extended β -sheet occurs next to the GX₆GX₃GG motif in subdomain II, highlighting the importance of this motif. In our structure, the Smchd1 hinge domain structure showed a closed conformation with both extended β -sheets intact (Figure 3A) [15]. It remains of outstanding interest whether the SMCHD1 hinge domain may similarly transition to open conformers, and how this might relate to its functions in epigenetic regulation.

Surface electrostatics of the Smchd1 hinge domain structure identified positive surfaces in the inter-subdomain linker region and in the central pore formed between the dimers (Figure 3E,F). In canonical SMC proteins positively charged residues can play a role in DNA binding [51,55]. This is also the case for SMCHD1, however, only positively charged residues buried in the central pore of the Smchd1 hinge domain were demonstrated to produce an altered phenotype in cellular experiments (Figure 3F) [15]. This includes the FSHD2

substitution arginine 1866² to glycine, which is part of the SMCHD1 GX₆GX₂GG motif, two amino acids after the first glycine (Figure 3F and Table 1). The other mutation that displayed an altered phenotype was arginine 1848 to alanine. Exactly how the SMCHD1 hinge domain interacts with chromatin and binds to DNA has not been established, but these data support a role for positively charged regions in the inter-subdomain linker and proximal to the central pore; how this may occur is discussed later.

Flanking the SMCHD1 hinge domain are two short coiled-coil regions. In the canonical SMC proteins, these coiled-coils are arranged head-to-tail and are intra-molecular. However, for Smchd1 this is unlikely to be the case and instead a head-to-head intermolecular arrangement is proposed [13,15,24]. Part of the N-terminal α -helix was included in the crystallised Smchd1 hinge domain sequence, but no coiled-coils were observed in the electron density [15]. The positioning of the hinge domain N- and C- termini did not support either coiled-coil arrangement: head-to-tail intra-molecular or head-to-head intermolecular. Negative stain electron microscopy images suggest a head-to-head arrangement of homodimeric full-length Smchd1. The observed particles are long (>40 nm) and thin with globular domains observable at each apex, which are likely to be the ATPase and hinge domain segregated at the N- and C-termini [13]. This suggests a head-to-head arrangement of hinge domain protomers within the Smchd1 homodimer, with intermolecular coiled-coils. This evidence is corroborated by SAXS, which showed an asymmetric SAXS envelope supporting this unusual SMC architecture for the non-canonical Smchd1 [24]. However, whether the N- and C-terminal α -helices can interact is not clear and therefore a complete understanding of how SMCHD1 forms a head-to-head arrangement and the role of these α -helices remains unclear.

Hypothetical models and open questions

UBL domains are generally found in E3 ubiquitin ligases or hold functions within ubiquitylation systems. For example, Parkin is an E3 ligase where its N-terminal UBL domain has been established as auto-inhibitory of its E3 ligase activity [56]. In the chromatin-modifying complex polycomb repressive complex 1 (PRC1), its core component, Bmi1 (PCGF4), which is required for H2AK119 ubiquitylation, also has a predicted ubiquitin-like fold [57]. This is suggested to undergo homo-oligomerisation, contributing to Bmi1 function in ubiquitylation [58]. Other studies show that Bmi1 directly interacts with Ring1B, the E3 ligase of the PRC1 complex, and together they bind the histone surface of nucleosome core particle to correctly orient the E2 ligase UbcH5c for H2A ubiquitylation [59]. Linking these findings to SMCHD1, recent work has reported that Smchd1's localisation to the inactive X chromosome is dependent on the ubiquitylated Histone 2A Lys119 (H2AK119ub) epigenetic mark. Removal of PRC1-mediated H2AK119ub leads to global loss of Smchd1 protein stability and genome-wide changes in gene silencing [60,61]. While a direct interaction between SMCHD1 and H2AK119ub has not been detected experimentally, it is likely that an adaptor protein that is yet to be identified may bridge this interaction. UBL domains are known to mediate a broad range of protein interactions in other systems. In the case of SMCHD1, we hypothesise that the UBL domain plays a role in its localisation to H2AK119ub-marked chromatin via specific protein interactions that are yet to be determined.

The use of human induced pluripotent stem cells has allowed the investigation of several SMCHD1 disease variants from BAMS or FSHD patients. These cells have provided a platform to test the ability of SMCHD1 to interact with the FSHD-related macro-repeat array, *D4Z4* [62]. This repeat array houses the gene for the transcription factor DUX4, the driver of FSHD, whose expression is normally silenced by SMCHD1 [11]. One FSHD-associated variant, Q193P located in the SMCHD1 ATPase domain, exhibited enhanced binding to DUX4 [62]. Interestingly, the same mutation was previously shown to abrogate Smchd1's ATPase activity *in vitro* [49]. This phenomenon was not observed in BAMS-associated variants or controls [49,62]. Additionally, there are many FSHD2-associated variations that result in non-sense mediated decay of *SMCHD1* mRNA transcripts [11]. Consequently, these variants show less SMCHD1 associated with the *D4Z4* macro-repeat array and also fail to silence the array. Collectively, these data suggest that ATP hydrolysis is required for SMCHD1 to release itself from chromatin (Figure 1D), and an inability to do so results in a failure of SMCHD1-dependent epigenetic silencing at target genes. A similar mechanism in which cohesin releases from DNA relies on its ATPase activity has been previously proposed [37]. This model is based on the observation that mutations that impaired the ATPase activity of one of the cohesin component proteins, Smc1, precluded DNA release. Taken together, this idea raises the possibility that SMCHD1 may require a 'sweet-spot' of ATPase activity for optimal

²Human numbering, 1867 in the mouse Smchd1 hinge domain sequence and crystal structure.

function, and too little or too much may be equally detrimental. This phenomenon is highlighted in FSHD- and BAMS-associated mutants where opposing effects on ATPase activity were observed [49].

Although it is established that the SMCHD1 hinge domain is critical for chromatin interactions, it is currently unclear exactly how the positive amino acids proximal to the central pore can play such an important role in these interactions. This region is too distal from the surface to interact directly, inferring that the hinge domain undergoes a large transition from the closed conformation observed in the Smchd1 hinge domain crystal structure to interact with chromatin. Adopting an open hinge domain conformation could expose this region and allow direct interactions with DNA and histone proteins. A similar model has recently been proposed for the human cohesin complex, where the hinge domain heterodimer was captured in an open conformation while bound to single-stranded DNA [54]. It is also unclear whether the α -helices that flank the hinge domain play a role in SMCHD1 chromatin interactions. Whilst experimental evidence reveals a head-to-head and tail-to-tail arrangement for these regions, they could potentially form head-to-tail interactions in response to a stimulus, such as DNA binding.

Almost half the SMCHD1 polypeptide is structurally and functionally uncharacterised, with the linker region between the ATPase and the start of the coiled-coil, N-terminal to the hinge domain, completely unstudied. Sequence analysis predicts a combination of β -sheet and disorder in this region, which is a stark contrast with the canonical SMC proteins which are entirely α -helical in the corresponding region. This linker region appears to be a unique feature of SMCHD1 and may hold clues to how it can convert chromatin interactions and ATP hydrolysis into gene silencing events.

Based on the evidence we have presented here, we propose that SMCHD1 is initially recruited to H2AK119ub-marked chromatin sites [60], and forms interactions with DNA via its hinge domain (Figure 1D). Interaction with DNA may trigger a conformational change in SMCHD1's hinge domain, potentially through the opening of the dimer interface, to expose buried residues (e.g. arginine 1866³). This conformational change could open the central linker region between the ATPase and hinge domain to allow DNA to reel through the SMCHD1 dimer. ATP-binding is then proposed to induce ATPase dimerisation, leading to domain-swapping of the UBL domain [14]. ATP hydrolysis can then allow the ATPases to disengage from chromatin and transition back to a monomeric form, resulting in a V-like structure of SMCHD1. This would enable SMCHD1 to then directly interact with promoter and enhancer regions of genes it regulates such as the *Pcdh* cluster [3]. Whether SMCHD1's presence at these sites promotes chromatin interactions either via a loop extrusion or a chromatin compaction mechanism, as demonstrated for SMC proteins and MORC1, respectively, to silencing gene expression [33,35,36,40] remains of ongoing interest (Figure 1C).

In conclusion, SMCHD1 is an important epigenetic modifier that is involved in silencing genes and is required for normal development. Missense variations in the *SMCHD1* gene alter its ability to silence genes and can result in the human diseases BAMS and FSHD, debilitating developmental diseases with no known treatments. Although much progress has been made into understanding SMCHD1 structure and function, many hypotheses remain to be investigated to better understand how this enigmatic protein can result in disease and, importantly, whether it can be exploited as a potential therapeutic target.

Perspectives

- *Highlight importance of the field:* SMCHD1 is an important epigenetic modifier that is involved in silencing genes for normal cellular function. Missense substitutions in the *SMCHD1* gene alter its ability to silence genes and can result in the debilitating human developmental diseases, BAMS and FSHD, with no known cures.
- *A summary of current thinking:* SMCHD1 silences genes by initially localising to H2AK119ub-marked chromatin and further interacting with DNA through its SMC hinge domain. This promotes long range chromatin interactions and excludes transcriptional regulators from these regions silencing gene expression in these regions.

³Human numbering, 1867 in the mouse sequence.

- *A comment on future directions:* The exact mechanism by which SMCHD1 exerts its silencing function is yet to be established and the precise functions of large regions of the protein are currently unknown. Future work will establish precisely how the individual domains of SMCHD1 work in concert to regulate gene expression and whether these functions can be modulated as therapies for FSHD and BAMS.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Abbreviations

BAMS, Bosma arhinia microphthalmia syndrome; CTCF, CCCTC-binding factor; FSHD, facioscapulohumeral muscular dystrophy; FSHD, facioscapulohumeral muscular dystrophy; PRC1, polycomb repressive complex 1; SAXS, small-angle X-ray scattering; SMC, structural maintenance of chromosomes; UBL, ubiquitin like.

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